INDUCTION OF OXIDATIVE STRESS RELATED RESPONSES IN ARABIDOPSIS THALIANA FOLLOWING URANIUM EXPOSURE

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Anthropogenic activities such as uranium mining and milling, metal mining and smelting and the phosphate industry, have caused environmental uranium contamination in many countries. To evaluate the impact of uranium on the environment, it is important to enlarge the scant information available on uranium toxicity effects in plants and unravel by which mechanisms plants respond to uranium stress. The main objective of this research concerns studying uranium toxicity effects on morphological and metabolic level in *Arabidopsis thaliana* plants and investigating the role of oxidative stress as a modulator during uranium stress. Therefore, oxidative stress related responses, together with general developmental alterations, were studied for *Arabidopsis thaliana* plants exposed for 1, 3 and 7 days to uranium concentrations ranging from 0.1 µM to 100 µM.

As *Arabidopsis thaliana* roots were directly exposed to uranium via the nutrient solution, uranium was readily taken up by the roots. Toxicity effects were immediately visible as exposure to the highest uranium concentration (100 µM) caused stunted roots, a decrease in fresh weight, a disrupted nutrient profile and a disturbed water balance indicating plants started to wilt. Exposure to the lower uranium concentrations (0.1 and 10 µM) on the other hand, resulted in a transient hormesis effect based on the transient increase of fresh weight. While effects at morphological and physiological level were visible at all uranium concentrations applied, oxidative stress related responses were only present at the highest uranium concentration (100 µM).

Plasma membranes of root cells are the first targets of oxidative damage and a possible fast role for NADPH oxidases as ROS producers during a uranium-induced early oxidative burst has been suggested based on the increase in *RBOHD* transcript levels. While potassium leakage can be an indication for membrane damage, the increased *LOX1* expression, already observed after the first day, can also point to fast lipoxygenase mediated lipid peroxidation resulting in an enhancement of precursors for signaling molecules such as jasmonates. As ROS can both cause cellular damage and function as signaling molecules during stress response, their presence needs to be tightly regulated. SOD is an important $O_2^{\bullet-}$ scavenging enzyme during the early oxidative burst as its capacity immediately increased which was accompanied by a simultaneous increase in *FSD1* transcript levels but a down-regulation for *CSD1* and *CSD2* expression. For the detoxification of hydrogen peroxide, the increase in *CAT1*

transcript levels was again a fast response while increased peroxidase capacities were observed at a later stage. Although the APX capacity increased, the ascorbate redox balance completely shifted towards its oxidized form and this could not be inverted by action of glutathione. The cellular redox balance of the roots was, also based on growth and morphological effects, completely disturbed by the highly toxic uranium concentration of 100 µM. Results for oxidative stress related responses in the roots are published in Vanhoudt et al. (2011a).

For *Arabidopsis thaliana* leaves, due to a very low root-to-shoot transfer factor, only low uranium concentrations were present in the leaves. Nevertheless, toxicity effects were visible after exposure to uranium concentrations ranging from 1 to 100 µM. On growth and development level, leaf fresh weight decreased after exposure to 100 µM uranium for 1 and 3 days and this decrease was after 7 days also visible for 1 and 10 µM uranium. In accordance with the roots, wilting of the plants was also demonstrated by the disturbed water balance of the leaves. Uranium uptake also disturbed the nutrient profile with an important decrease in calcium and magnesium due to the competition with the uranyl ion for their binding sites.

As in leaves several responses were already visible after 1 day when uranium concentrations were negligible in the leaves and no proof was found for an early oxidative burst, oxidative stress was probably generated via other mechanisms such as root-to-shoot signaling. While an increase of membrane damage was indicated by the increase in TBA-reactive compounds and potassium leakage after 100 µM uranium, also an enhancement in signaling molecules was proposed by LOX induced lipid peroxidation for which a transient concentration dependent response pattern was visible. This transient character, both in time as with concentration, of leaf responses to uranium stress was emphasized by *LOX2* transcript levels, antioxidative enzyme capacities and gene expression and glutathione concentrations. The ascorbate pool on the other hand continuously increased in a concentration and time dependent way characterized by an increase of the AsA/DHA balance towards its reduced form. The increase and maintenance of the ascorbate redox balance is an important response for hydrogen peroxide detoxification or a response in signaling functions. In addition to the several fast transient effects, the increase in ascorbate could represent either a slow transient response or a stable increase with regard to plant acclimation to uranium. However this can only be assumed for the lower uranium concentrations as 100 µM uranium toxicity effects in the roots are too severe to allow plant survival. Results for oxidative stress related responses in the leaves are published in Vanhoudt et al. (2011b).

In conclusion, 100 µM uranium is extremely toxic for *Arabidopsis thaliana* plants with a completely inhibited growth, a fully disturbed nutrient profile, wilting and although making an effort to increase the antioxidative defense, suffering from severe oxidative stress with a completely disturbed metabolic balance. While at lower uranium concentrations no oxidative stress related responses are visible in the roots, leaves show an increased defense against uranium stress with an important regulatory role for the ascorbate pool as a stable stress response mechanism.

References

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